HA Tag CUTANA™ CUT&RUN Antibody

Catalog No	13-2010	**Type**	Polyclonal
Lot No	24294004-81	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	1,000 µg/mL
Applications	CUT&RUN, WB	**Reactivity**	HA Epitope (YPYDVPDYA)

DESCRIPTION

This antibody meets EpiCypher's "CUTANA Compatible" criteria for performance in Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and/or Cleavage Under Targets and Tagmentation (CUT&Tag) approaches to genomic mapping. Every lot of a CUTANA Compatible antibody is tested in the indicated approach using EpiCypher optimized protocols (epicypher.com/protocols) and determined to yield peaks that show a genomic distribution pattern consistent with reported function(s) of the target. HA antibody is useful for studies utilizing HA-tagged target proteins. HA Tag antibody produces CUT&RUN peaks (**Figure 2**) in breast cancer cells expressing 3xHA-tagged GATA3 transcription factor [1]*.

**Thanks to Dr. Takaku (UND) for 3xFLAG-GATA3-3xHA MDA-MB-231 cells.*

TECHNICAL INFORMATION

Immunogen	A synthetic HA peptide (sequence: YPYDVPDYA)
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Antigen affinity-purified antibody in phosphate buffered saline (PBS), 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction	**Western Blot**	1:1,000 - 1:30,000

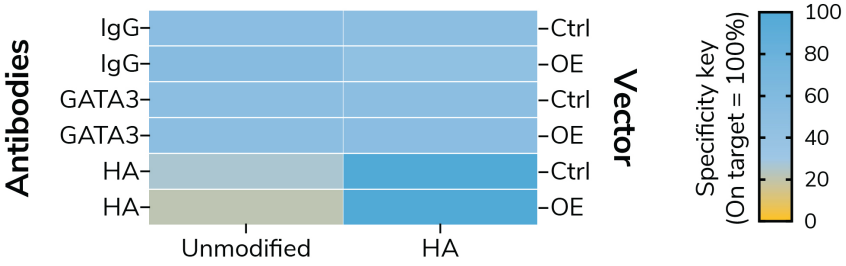
REFERENCES

[1] Takaku et al. *Genome Biol.* (2016). PMID: 26922637

VALIDATION DATA

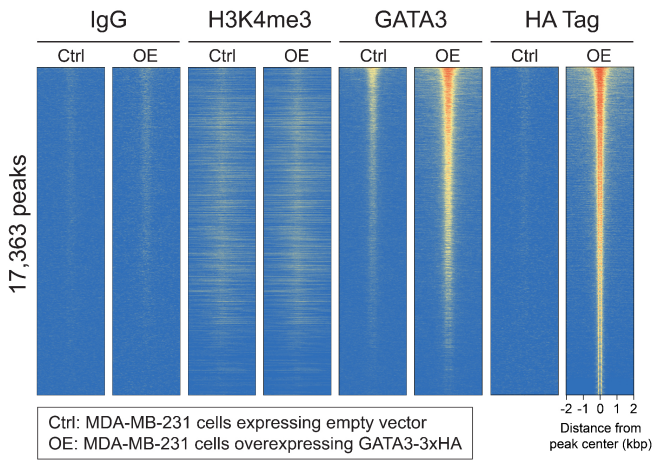
CUT&RUN Methods

CUT&RUN was performed on 500k MDA-MB-231 native cells stably overexpressing c-terminal 3xHA-tagged GATA3 [1] or containing vector control. 0.5 µg of either HA Tag, H3K4me3 (EpiCypher 13-0060), GATA3 (CST 5852), or IgG (EpiCypher 13-0042) antibodies were used with the CUTANA™ ChIC/CUT&RUN Kit v4 (EpiCypher 14-1048). Library preparation was performed with 5 ng of DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ Library Prep Kit (EpiCypher 14-1001/14-1002). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth (vector control cells/overexpressing cells) was 4.7/6.3 million reads (IgG), 1.6/2.5 million reads (H3K4me3), 5.6/7.1 million reads (GATA3), and 2.5/3.5 million reads (HA Tag). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.



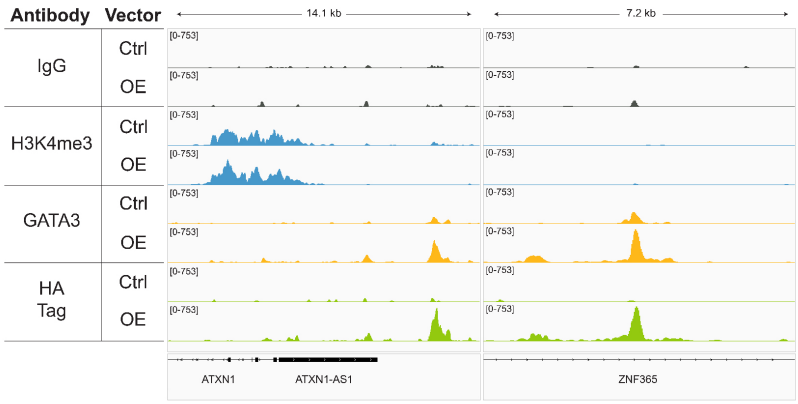
Ctrl: MDA-MB-231 cells expressing empty vector
OE: MDA-MB-231 cells overexpressing GATA3-3xHA

FIGURE 1 Defined nucleosome spike-ins provide an in-assay control for HA Tag antibody in CUT&RUN. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the SNAP-CUTANA™ HA Tag Panel (EpiCypher 19-5002). Data are expressed as a percent relative to on-target recovery (HA Tag set to 100%) or total counts (IgG/GATA3). IgG/GATA3 show no preferential binding to unmodified or HA spike-in nucleosomes. HA Tag antibody selectively enriches the HA Tag spike-in nucleosome, validating the antibody in CUT&RUN.



Ctrl: MDA-MB-231 cells expressing empty vector
OE: MDA-MB-231 cells overexpressing GATA3-3xHA

FIGURE 2 HA-tagged protein peaks in CUT&RUN. Heatmaps show HA Tag antibody-enriched peaks called for GATA3-3xHA overexpressing cells (OE) in aligned rows relative to all other experimental conditions. Rows are ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. A high degree of overlap is observed for HA and GATA3 antibodies as expected in the OE cells, while empty vector control (Ctrl) shows absence of HA and lower GATA3 enrichment representing endogenous protein. IgG shows low background signal. H3K4me3, a canonical mark of promoters, does not appear in regions of high GATA3 enrichment.



Ctrl: MDA-MB-231 cells expressing empty vector
OE: MDA-MB-231 cells overexpressing GATA3-3xHA

FIGURE 3 CUT&RUN representative browser tracks for HA-tagged protein. Gene browser shows were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two of the top called peaks in GATA3-3xHA overexpressing (OE) cells are shown. The peaks show the same distribution patterns as observed in the genome-wide heatmaps (**Figure 2**). IgG shows low background, H3K4me3 is unchanged between empty vector control (Ctrl) and OE cells, and peaks overlap between GATA3 and HA antibodies in OE cells. These results demonstrate the robustness and specificity of HA Tag antibody in CUT&RUN experiments targeting HA-tagged proteins.

VALIDATION DATA

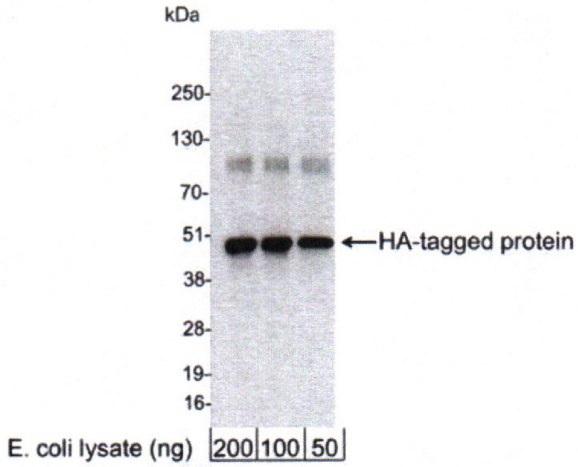


FIGURE 4 **Western blot data**. *E. coli* cells expressing a multi-tag fusion protein were used to prepare whole cell lysates. The indicated amounts (ng) of lysate were loaded onto a 4-20% SDS-PAGE gel and analyzed under standard western blot conditions using HA Tag antibody at a dilution of 1:25,000.